

Michael I. Watkins et al.
Application No.: 09/548,883
Page 2

- (i) particles coated with anti-thyroid stimulating hormone,
- (ii) particles coated with anti-triiodothyronine,
- (iii) particles coated with anti-thyroxine, and
- (iv) particles coated with a mixture of a diluting agent and a member selected from the group consisting of thyroid peroxidase and anti-human IgG,

the particles of each group distinguishable from the particles of each other group by a flow cytometry distinguishable characteristic that is independent of the coatings of subparagraphs (i), (ii), (iii), and (iv);

- (b) recovering said particles from said first suspension, and incubating said recovered particles with a mixture of labeled binding members capable of binding to the recovered particles in a second suspension, said mixture of labeled binding members comprising:

- (1) labeled anti-thyroid stimulating hormone,
- (2) a labeled analog composition toward which anti-triiodothyronine and anti-thyroxine have immunological binding affinity, but in which said immunological binding affinity is less than that of anti-triiodothyronine toward triiodothyronine and of anti-thyroxine toward thyroxine, and
- (3) either labeled anti-human IgG when particles of group (iv) are coated with thyroid peroxidase, or labeled thyroid peroxidase when particles of group (iv) are coated with anti-human IgG;

said diluting agent being inert toward said biological markers and said labeled binding members; and

- (c) recovering said particles from said second suspension and detecting the amount of label bound to said particles thus recovered from said second suspension while correlating by flow cytometry the amount of label thus detected to the group to which said label is bound, thereby simultaneously obtaining values individually representative of the

b1

1 encl
levels of thyroid stimulating hormone, triiodothyronine, thyroxine, and
anti-thyroid peroxidase.

2 12. (Amended) A method in accordance with claim 1 in which said
particles include dyes, each of groups (i) through (iv) including a distinct dye that is
distinguishable by flow cytometry over the dyes of each other group, and step (c)
comprises distinguishing such dyes by flow cytometry while detecting the amount of
label bound to said particles.

20. (Amended) A method in accordance with claim 1 in which group (i)
is comprised of two subgroups differing from each other by particle size such that one
subgroup provides a substantially greater sensitivity for measuring lower concentrations
of TSH, than the other.

21. (Amended) A method in accordance with claim 1 in which group (i)
is comprised of two subgroups differing from each other by coating density of
anti-thyroid stimulating hormone such that one subgroup provides a substantially greater
sensitivity for measuring lower concentrations of TSH, than the other.

3 22. (Amended) A method in accordance with claim 1 in which group (i)
is comprised of two subgroups differing from each other by both particle size and coating
density of anti-thyroid stimulating hormone such that one subgroup provides a
substantially greater sensitivity for measuring lower concentrations of TSH, than the
other.

Add the following new claims:

4 26. A method according to claim 20 in which one group of particles
provides a greater sensitivity for measuring lower concentrations of TSH than the other,
and the second subgroup of particles provides a greater sensitivity for measuring higher
concentrations of TSH than the other.

27. A method according to claim 21 in which one group of particles provides a greater sensitivity for measuring lower concentrations of TSH than the other, and the second subgroup of particles provides a greater sensitivity for measuring higher concentrations of TSH than the other.

28. A method according to claim 22 in which one group of particles provides a greater sensitivity for measuring lower concentrations of TSH than the other, and the second subgroup of particles provides a greater sensitivity for measuring higher concentrations of TSH than the other.

29. A composition for analyzing a single sample to simultaneously determine levels of four biological markers indicative of thyroid disorders, said composition comprising a mixture of particles comprised of groups (i) through (iv):

- (i) particles coated with anti-thyroid stimulating hormone,
- (ii) particles coated with anti-triiodothyronine,
- (iii) particles coated with anti-thyroxine, and
- (iv) particles coated with a mixture of a diluting agent and a member selected from the group consisting of thyroid peroxidase and anti-human IgG,

the particles of each group distinguishable from the particles of each other group by a flow cytometry distinguishable characteristic that is independent of the coatings of subparagraphs (i), (ii), (iii), and (iv).

30. A test system for analyzing a single sample to simultaneously determine levels of four biological markers indicative of thyroid disorders, said test system comprising

(a) a mixture of particles comprised of groups (i) through (iv):

- (i) particles coated with anti-thyroid stimulating hormone,
- (ii) particles coated with anti-triiodothyronine,
- (iii) particles coated with anti-thyroxine, and

(iv) particles coated with a mixture of a diluting agent and a member selected from the group consisting of thyroid peroxidase and anti-human IgG, the particles of each group distinguishable from the particles of each other group by a flow cytometry distinguishable characteristic that is independent of the coatings of subparagraphs (i), (ii), (iii), and (iv); and

(b) a mixture of label binding members comprising:

- (1) labeled anti-thyroid stimulating hormone,
- (2) a labeled analog composition toward which anti-triiodothyronine and anti-thyroxine have immunological binding affinity, but in which said immunological binding affinity is less than that of anti-triiodothyronine toward triiodothyronine and of anti-thyroxine toward thyroxine, and
- (3) either labeled anti-human IgG when particles of group (iv) are coated with thyroid peroxidase, or labeled thyroid peroxidase when particles of group (v) are coated with anti-human IgG; said diluting agent being inert toward said biological markers and said labeled binding members.

REMARKS

Newly added claims 26-28 are supported in the specification at page 10. New claims 29 and 30 are supported by the specification at pp. 2-8 and elsewhere. The specification does not contain the exact language of a "test system"; however, it is clear from the specification that one aspect of this invention is a combination of the differentiable particles with their specific coatings and a composition comprising the stated labeled binding members as described, for instance on p. 6. Should the examiner